One polymorphism at the Stearoyl CoA Desaturase (SCD) gene is associated to CLA content of sheep milk fat

Sabrina Miari, Mario Graziano Usai, Tiziana Sechi, Annalisa Pernisa, Antonello Carta

Settore Genetica e Biotecnologie - DIRPA, AGRIS-Sardegna, Olmedo, Italy

Corresponding author: Sabrina Miari. Settore Genetica e Biotecnologie - DIRPA, AGRIS-Sardegna. Loc. Bonassai SS291 km 18.6, 07040 Olmedo (SS), Italy - Tel. +39 079 3750378 - Fax: +39 079 389450 - Email: sabrinamiari@gmail.com

ABSTRACT - On OAR 22 a highly significant QTL affecting the rate of desaturation of vaccenic acid (VA) to conjugated linoleic acid (CLA) in the mammary gland of sheep was found segregating on four dairy sheep families of a Sardinian x Lacaune back-cross population. The most likely location of the QTL was 35 cM corresponding to Stearoyl CoA Desaturase (SCD) gene location on the Australian Sheep Gene Map. The aim of this work was finding mutations along the SCD gene associated with the CLA/VA variability in sheep milk. The sequencing of a large part of the gene in the four informative sires allowed identifying one SNP in intron 4 (3295 C>T relative to *Capra hircus* SCD gene, GenBank Acc. no. AH011188). The sequencing of high and low phenotypic tails' progeny from the most significant family showed a strong association of the found mutation with the CLA/VA variability.

Key words: QTL, CLA, SCD, SNP.

Introduction – The fatty acid (FA) content of milk fat affects the technological properties and the nutritional value of dairy products. Sheep cheese is highly appreciated for its taste and flavour but is commonly considered potentially negative for human health due to high content of fat matter. The saturated FA (SFA) level in sheep milk fat is higher than 60%, while monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) are approximately 28% and 6%, respectively. Recently, particular interest has been paid to conjugated linoleic acid (CLA), due to its beneficial effects on human health. The generic name CLA embraces all octadecadienoic acids ($C_{18:2}$) with a conjugated double bond system. The most important isomer in milk fat of ruminants corresponds to cis-9 trans-11 (rumenic acid) that represents more than 75% of total CLA. A portion of CLA is formed in the rumen by biohydrogenation of linoleic acid, escapes further biohydrogenation, and is absorbed in the digestive tract (Griinari and Bauman, 1999). The extent of this process is minimal, while the intermediate product of CLA biohydrogenation (trans 11-$C_{18:1}$; vaccenic acid) accumulates. The other important source of VA in the rumen is the biohydrogenation of the linolenic acid ($C_{18:3}$). This pathway does not have as intermediate CLA. VA produced in the rumen is desaturated to produce CLA in the mammary gland tissues by Stearoyl CoA Desaturase (SCD). There is evidence that milk CLA basically comes from this pathway. Few studies are available on the genetic determinism of this trait. This is mainly due to the fact that large-scale phenotyping is needed to produce accurate genetic studies and, that until recently, the only available technique has been very time-consuming and costly gas chromatography technique. However, Soyeurt *et al.* (2006) showed that genetic variation exists across and within cattle breeds for FA profile of milk, using a mid-infrared spectrometry technique which is less laborious and expensive. Recent studies highlighted a relationship between SCD gene polymorphism and milk production traits (Macciotta *et. al.*, 2008) and with FA composition (Mele *et al.*, 2007) in Italian Holsteins. In dairy sheep, Carta *et al.* (2008) us-

ing a backcross Sardinian x Lacaune population data, estimated important individual variances and a high number of QTL affecting most FA and their combinations or ratios. In particular, a highly significant QTL on the ovine chromosome 22 affecting the rate of desaturation of VA to CLA in the mammary gland of sheep was found segregating on four out of the ten analysed families. The most likely location of the QTL was 35 cM corresponding to the position of the SCD gene on the available maps (Maddox, Australian Sheep Gene Mapping Web Site 2007). Based on these results, the aim of this work was finding mutations along this gene associated with the CLA and VA ratio variability in sheep milk.

Material and methods – Primer pairs to amplify SCD ovine gene were designed using Primer 3 software (http://frodo.wi.mit.edu/). Since only the ovine SCD coding region was reported in literature (Ward *et al.*, 1998, GenBank Acc. no. NM_001009254), most of primers were designed using as template the caprine one. Only small portions of the coding region were sequenced using ovine primers. For the promoter region, neither ovine nor caprine sequences were available and so primers were designed using as template the bovine one. Amplified products were sequenced in both directions with ABI PRISM Big Dye TerminatorCycle Sequencing Ready Reaction v.3.1 (Applied Biosystems, Foster City CA, USA). Sequencing was carried out using an ABI PRISM® 3100- Avant Genetic Analyzer (Applied Biosystems). Data were collected using the ABI PRISM Data collection software and analyzed with DNA Sequencing Analysis software v3.7 (Applied Biosystems). The sequences were then edited and aligned with caprine SCD gene and bovine promoter (GenBank Acc. no. AH011188; AY241932) using Sequencher software v.4.8 (Gene Codes Corporation, USA). In a first step, SCD gene was sequenced in the four informative sires of the Sardinian x Lacaune population with the aim of detecting SNP at the heterozygous state. Then animals in the high and low tails of the distribution of the ratio between CLA and VA ratio in the progeny of informative sires were also sequenced to verify the transmission disequilibrium of the found SNP.

Results and conclusions – The whole coding region (6 exons), the 5'-UTR and 3'-UTR region, the 1, 3, 4, and 5 introns were completely sequenced. In intron 2, 260 bp were sequenced whereas the remaining portion

Table 1. Marker haplotypes, SNP genotype (G) and phenotypic value (P) of daughters in high and low tails from the only sire polymorphic at SNP in intron 4. Blank indicates that it was not possible to identify the marker allele transmitted by the sire.

High tail					Low tail				
HEL11	INRA81	BMS332	G	P	HEL11	INRA81	BMS332	G	P
176	152		C/T	6.29	170	176	146	C/C	-5.28
176	152	140	C/T	5.17	170	176	146	C/C	-4.78
	152	140	C/T	4.80	170		146	C/C	-4.61
176	152	140	C/T	4.72	170	176	146	C/C	-4.40
176	152		C/T	4.57	170	176	146	C/C	-4.24
176	152	146	C/T	4.38	170	176	146	C/C	-4.13
176	152	140	C/T	3.61	170	176	146	C/C	-4.03
176		140	C/T	3.47	176	176	146	C/C	-3.36
170	176	146	C/C	2.90	170	176	146	C/C	-3.21
176	152		C/T	2.50	176	152		C/T	-3.03
176	152	140	C/T	2.25	170	176		C/C	-2.68
176	152	146	C/T	1.64	170	176	146	C/C	-2.33
176		146	C/T	1.53	176	176		C/C	-2.11

is still to be sequenced. In the promoter region, 1700 bp, including 374 bp of region upstream of the transcription start site, were sequenced. The length of the promoter region in bovine was 1880 bp whereas it was not available in caprine. In the whole, approximately 9 kb of the SCD gene were sequenced. Currently the primer designing to complete sequencing of ovine SCD gene is ongoing basing on the sheep genome sequences available on the Australian Sheep Gene Mapping Web Site (2008).

The sequencing of this large part of the gene allowed to identify one SNP in ovine intron 4 (3295 C>T relative to *Capra hircus* SCD gene; Miari, 2008). This polymorphism was detected only on the most significant sire among the four analysed (LRT=29.13; allelic substitution effect=1.04 residual standard deviation units). Twenty six among the 64 daughters of this sire (13 out of 14 with the lowest and 13 out of 14 with the highest phenotypes) were sequenced to detect the SNP allele transmitted by the sire. Table 1 shows the alleles transmitted by the sire to the daughter at the found SNP and at 3 microsatellites surrounding the most probable location of the SCD gene (HEL11, 30 cM; INRA81, 35.7 cM; BMS332, 36.9 cM). The previous QTL detection analysis allowed determining the marker haplotypes with the positive and negative effect respectively. Out of 13 animals of the high tail, 12 inherited the T SNP allele (C/T genotype) whereas only one animal inherited the C allele (C/C genotype). The chromosome segment was interested by 3 recombination events regarding BMS332. The T allele was always linked to positive marker haplotypes, whereas the C allele was linked to the paternal marker haplotype. Out of the 13 animals of the low tail, 12 inherited the C allele (C/C genotype) whereas only one animal inherited the T allele (C/T genotype). The chromosome segment was interested by 2 recombination events regarding HEL11. The C allele was always linked to negative marker haplotypes whereas the T allele was associated with the paternal positive marker haplotype. As a whole, these results confirm a strong association between the found SNP and the phenotypic value of CLA to VA ratio. However, the SNP found is in a non-coding region and, thus, the most likely conclusion is that the mutation found is in strong linkage with the causative one. This study will be completed by sequencing the remaining part of the SCD ovine gene to verify if there are additional SNP having a structural or regulatory role. In particular it will be essential to complete the sequencing of the promoter region since it might affect the expression level. Although this study did not allow for the immediate application of Marked Assisted Selection or Gene Assisted Selection, the high significance of the QTL found clearly indicates that this is a very crucial genome region to increase CLA content in sheep milk fat.

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REFERENCES – Carta, A., Casu, Sara, Usai, M.G., Addis, M., Fiori, M., Fraghì, A., Miari, S., Mura, L., Piredda, G., Schibler, L., Sechi, T., Elsen, J.M., Barillet, F., 2008. Investigating the genetic component of fatty acid content in sheep milk. Small Rumin. Res.79:22-28. **Griinari,** J.M., Barman, D.E., 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: MP. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza, and G.J. Nelson (Ed.). Advances in Conjugated linoleic Acid Research, Vol. 1. pp 180-200. AOCS Press, Champaign, IL. **Macciotta**, N.P.P., Mele, M., Conte, G., Serra, A., Cassandro, M., Dal Zotto, R., Cappio Borlino, A., Pagnacco, G., Secchiari, P., 2008. Association between a polymorphism at the Stearoyl CoA Desaturase locus and milk production traits in Italian Holsteins J. Dairy Sci. 91:3184-3189. **Mele**, M., Conte G., Castiglioni, B., Chessa, S., Macciotta, N.P.P, Serra, A., Buccioni, A., Pagnacco, G., Secchiari, P., 2007. Stearoyl-Coenzyme A Desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. J. Dairy Sci. 90:4458-4465. **Miari**, S., 2008. Association between ovine Stearoyl-CoA desaturase gene polymorphisms and the Conjugated Linoleic Acid content in sheep milk fat: results of a positional candidate gene study. PhD Thesis, Università degli Studi di Sassari. **Soyeurt**, H., Dardenne, P., Dehareng, F., Lognay, G., Veselko, D., Marlier, M., Bertozzi, C., Mayeres, P., Gengler, N., 2006. Estimating fatty acid content in cow milk using mid-infrared spectrometry. J. Dairy Sci. 89:3690-3695. **Ward,** R.J., Travers, M.T., Richards, S.E., Vernon, R.G., Salter, A.M., Buttery, P.J., Barber, M.C., 1998. Stearoyl-CoA desaturase mRNA is transcribed from a single gene in the ovine genome. Biochim. Biophys. Acta. 1391:145-156.